## THERAPEUTIC USE OF D-METHIONINE
## TO REDUCE THE TOXICITY OF NOISE

This patent application is a continuation-in-part of
recently allowed U.S. Patent Application Serial Number
09/057,065, filed April 8, 1998, which is a continuation-in-
part of U.S. Patent No. 6,187,817.  The entire contents of
U.S. Patent Application Serial Number 09/057,065 and U.S.
Patent No. 6,187,817 are hereby incorporated herein by
reference.

## Background of the Invention
## Field of the Invention

The present invention relates to the use of protective
agents in cancer chemotherapy in human and animal subjects.
Protective agents are compounds that prevent, reduce, or
otherwise ameliorate the toxic side effects of anti-cancer
chemotherapeutic compounds in normal body cells while
substantially preserving the anti-tumor properties of these
compounds *in vivo* when administered prior to, concomitantly
with, or subsequently to administration of such
chemotherapeutic compounds.  More specifically, the present
invention relates to the use of D-methionine and
structurally related compounds as protective agents having
oto-protective, weight loss-protective, gastrointestinal-
protective, neuro-protective, alopecia-protective, and
survival-enhancing effects in conjunction with chemotherapy
employing platinum-containing anti-neoplastic agents, such
as cisplatin.  The present invention also relates to the use
of D-methionine and structurally related compounds as
protective agents having oto-protective effects against
noise-induced, loop diuretic-induced, aminoglycoside
antibiotic-induced, iron chelator-induced, quinine- and
quinidine-induced, and radiation-induced hearing loss, as
well as protective effects in ameliorating other radiation-

induced side effects such as neural damage, alopecia,
gastrointestinal disorders, and reduced patient survival.


## Description of Related Art

### Cisplatin Chemotherapy

5          Cisplatin (*cis*-diamminedichloroplatinum(II); CDDP) is a
widely used antineoplastic agent.   Cisplatin administration
has increased both in the variety of cancer types for which
it is employed and in the amount used in a given individual
to achieve maximal therapeutic effect (Blumenreich et al.,
10       1985; Forastiere et al., 1987; Gandara et al., 1989).

        The toxic side effects of cisplatin have long been
recognized and are widely reported (Lippman et al., 1973;
also see the review by Hacker, 1991).   These toxicities
include a variety of peripheral neuropathies, myelo-
15       suppression, gastrointestinal toxicity, nephrotoxicity, and
ototoxicity (Ozols and Young, 1985; Stewart et al., 1987;
Stoter et al., 1989).   Initially, the primary dose-limiting
factor was nephrotoxicity, but now the routine
administration of mannitol, hypertonic saline, and high
20       fluid administration have ameliorated, but not eliminated,
that side effect.   However, ototoxicity remains uncontrolled
(Bajorin et al., 1987; Fillastre and Raguenez-Viotte, 1989).
Although nephrotoxicity can still be dose-limiting,
currently the primary dose-limiting factor is ototoxicity
25       (Blumenreich et al., 1985; Forastiere et al., 1987; Berry et
al., 1990).

        The primary ototoxic effects of cisplatin appear to
occur in the cochlea.   Anatomical changes occur in both the
stria vascularis and the organ of Corti.   The primary
30       histologic findings include hair cell degeneration and
damage to the supporting cells that are dose-related (Anniko
and Sobin, 1986).   At high doses, total collapse of the
membranous labyrinth can occur (Anniko and Sobin, 1986).   In

the organ of Corti, there is loss of outer and inner hair cells, with a propensity for outer hair cell loss in the basal turn (Fleischman et al., 1975; Komune, 1981; Estrem et al., 1981; Schweitzer, 1993), and alterations in the supporting cells and Reissner's membrane (Komune, 1981; Estrem et al., 1981). Estrem et al. (1981) also reported softening of the cuticular plate and an increased number of lysosomal bodies in the apical portion of the outer hair cell. However, the mechanisms inducing these changes are largely unknown.

For equivalent inner ear concentrations, cisplatin is the most ototoxic drug known (Moroso and Blair, 1983; Koegel, 1985; Anniko and Sobin, 1986; Griffin, 1988). Generally, cisplatin ototoxicity is irreversible, its onset insidious, and the hearing loss may progress after discontinuation of the protocol (Schaefer et al., 1985; Melamed et al., 1985; Pollera et al., 1988; Aguilar-Markulis et al., 1981; see the review by Moroso and Blair, 1983). Hearing loss is usually permanent (Vermorken et al., 1983). Partial recovery may occur in some cases, but only one of 121 patients with hearing loss had complete recovery in a study by Aguilar-Markulis et al., (1981). Hearing loss typically starts at the ultra high frequencies (9000 to 20000 Hz) (Fausti et al., 1984; Kopelman et al., 1988) and then progresses into the high conventional audiometric range (Laurell and Engström, 1989; Kopelman et al., 1988; Meyer, 1989), reducing the patient's ability to hear consonant but not vowel sounds. An inability to understand speech and tinnitus are frequent complaints (Kopelman et al., 1988). An increasing number of patients survive chemotherapy, but frequently with hearing impairment.

**Nucleophilic Sulfur Protective Agents**

Many sulfur-containing compounds (including substances with thio, thiol, and thioether groups) have been reported to provide CDDP nephroprotection in animal models (Anderson et al., 1990; Jones and Basinger, 1989; Jones et al., 1986; 1991a, b, c; 1992). These compounds may act by preventing the CDDP-induced depletion of glutathione or the binding of CDDP to protein sulfhydryl groups (Hanneman and Baumann, 1988; Nakano and Gemba, 1989; Gandara et al., 1989; Ravi et al., 1991; Schweitzer, 1993).

Additionally, sodium thiosulfate (STS) and diethydithiocarbamate (DDTC) provide good CDDP otoprotection in animals (Otto et al., 1988; Church et al., 1995; Rybak et al., 1995). Unfortunately, STS may reduce CDDP tumoricidal action (Pfeifle et al., 1985; Aamdal et al., 1987) and may exacerbate CDDP-induced weight loss and mortality (Otto et al., 1988). DDTC does not interfere with antitumor action (Qazi et al., 1988; Berry et al., 1989; Dedon et al., 1984; Borch et al., 1988), but can produce severe side effects (Rothenberg et al., 1988; Qazi et al., 1988).

**D-Methionine**

D-methionine (D-Met) is a sulfur-containing nucleophile that provides highly effective CDDP nephroprotection in animals without decreasing anti-tumor action (Jones and Basinger, 1989). Although only tested in that single study at a single dose level, D-Met was the most effective CDDP nephroprotectant that did not interfere with CDDP tumoricidal action out of nearly 40 sulfur-containing agents tested in a series of studies by Jones and colleagues (Jones and Basinger, 1989; Jones et al., 1986; 1991a, b, c; 1992). As far as the inventor is aware, D-Met has never been previously tested as a CDDP otoprotectant, and has not yet been tested clinically (Treskes and van der Vijgh, 1993).

## Sulfur-Containing Protective Agents and the Modulation of Cisplatin-Induced Toxicity

Studies indicate that individual sulfur-containing protective agents may only be effective in reducing specific types of toxicity, such as nephrotoxicity, while remaining ineffective in blocking other platinum-related complications such as peripheral neuropathy and ototoxicity (Schweitzer, 1993). In addition, an agent which is effective as a regional chemoprotector following site-specific (intraperitoneal) usage of platinum-containing compounds such as CDDP may fail to provide adequate systemic protection, or may inhibit antitumor activity (Schweitzer, 1993).

Not all sulfur-containing compounds provide protection against all of CDDP's toxicities, and it is not possible to predict which protective agents will be effective or ineffective for this purpose. For example, cefoxitin (Jones et al., 1992) does not provide nephroprotection. Ethyl-L-cysteinate and N-(2-mercapto- propionyl)glycine (Jones and Basinger, 1989) exacerbate CDDP nephrotoxicity. 2-(methylthio)nicotinic acid does not provide nephroprotection in rats (Jones et al., 1991b). The sodium salt of penicillin G does not protect against CDDP nephrotoxicity or weight loss (Jones et al., 1992). Similarly, thiamine-HCl does not protect against cisplatin nephrotoxicity or weight loss (Jones et al., 1992).

Furthermore, sulfur-containing compounds protective against one type of CDDP toxicity frequently do not protect against other CDDP toxicities, and it is not possible to predict the specific antitoxic effectiveness of such compounds. Cephalexin (Jones et al., 1992) protects against CDDP-induced kidney dysfunction and weight loss, but curiously does not prevent kidney pathology. Cefoxitin (Jones et al., 1992) provides some protection against CDDP-induced weight loss, but does not protect against CDDP

nephrotoxicity.  The sodium salt of penicillin G does not
protect against either CDDP-induced nephrotoxicity or weight
loss (Jones et al., 1992).  Sulfathiazole provides
protection against CDDP nephro- toxicity, but not weight
loss (Jones et al., 1992).

WR2721 provides excellent CDDP nephroprotection
(Mollman et al., 1988), but does not ameliorate nausea and
vomiting (Glover et al., 1987).  Nor does WR2721 seem to
provide CDDP otoprotection: Glover et al. (1987) found mild
to severe hearing loss in 20 of 36 patients receiving WR2721
prior to CDDP although nephroprotection was obtained.  Rubin
et al. (1995) reported a 45% incidence of significant
hearing threshold shift in patients pretreated with WR2721
prior to CDDP administration.  Unfortunately, neither the
Glover et al. (1987) nor Rubin et al. (1995) studies
employed a control group, and both reported a high incidence
of ototoxicity in patients receiving WR2721.  In hamsters,
Church et al. (1995) reported no WR2721 protection from
ototoxicity or mortality.

Even when a sulfur-containing agent is found to be
protective, its side effects can be so severe that clinical
applicability is precluded.  In addition, even among agents
that provide CDDP otoprotection, the protection may be so
inconsistent and/or the side effects so great that they
would not be used clinically.  For example, DDTC provides
protection against CDDP-induced nephrotoxicity (Qazi et al.,
1988; Berry et al., 1989; Gandara et al., 1989a, 1989b, and
1991) and ototoxicity (Church et al., 1995), but the
protection against ototoxicity may only be partial (Gandara
et al., 1989a; Ravi et al., 1992) and its side effects are
severe (Rothenberg et al., 1988; Berry et al., 1990).  If
DDTC dosing is reduced to ameliorate its side effects,
adequate protection from CDDP side effects may not occur
(Paredes et al., 1988).  Similarly, disulfiram (Antabuse),

which can be used as a precursor for its metabolite DDTC,
can cause sensorimotor neuropathy (Argov and Mastiglia,
1979) and reversible confusion that can be dose-limiting
(Stewart et al., 1987). Consequently, it is unlikely that
DDTC will be widely used clinically as a CDDP
chemoprotectant. In contrast, as described below, D-Met
provides complete otoprotection without apparent adverse
side effects.

Finally, many sulfur-containing compounds inhibit the
anti-tumor action of CDDP, and it is not possible to predict
which agents will or will not act in this manner. Thus,
many agents that provide CDDP protection are not clinically
useful. For example, Captropril (Jones et al., 1992)
protects against CDDP nephrotoxicity, but reacts immediately
with CDDP to form a precipitate if coadministered, thereby
precluding anti-tumor efficacy. L-methioninamide (Jones et
al., 1991b) provides excellent CDDP nephroprotection, but
impairs CDDP anti-tumor action. Metallothionein, a sulfur-
containing compound the synthesis of which is induced by
administration of bismuth subnitrate, provides CDDP
nephroprotection, but also inhibits CDDP anti-tumor action
(Naganuma et al., 1987; Boogaard et al., 1991; Satoh et al.,
1993; Imura et al., 1992; Endresen et al., 1994). STS
reduces CDDP nephrotoxicity (Pfeifle et al., 1985; Howell et
al. 1982) and ototoxicity (Otto et al., 1988; Church et al.,
1995), although some authors report inadequate otoprotection
(Markman et al., 1985). However, STS will probably not be
clinically useful as coadministration with CDDP reduces the
latter's tumoricidal action (Pfeile et al., 1985; Aamdal et
al., 1987; Jones et al., 1991b), and two route
administration does not provide nephroprotection (Jones et
al., 1991b). Even in the absence of other agents, STS may
also increase mortality and induce weight loss (Otto et al.,
1988). Biotin, another sulfur-containing compound that

provides good CDDP nephroprotection, inhibits anti-tumor
activity (Jones et al., 1992).

Thus, a variety of sulfur-containing compounds can act
as protective agents for particular toxicities. A
comparison of C-SH- and C-S-C-containing compounds
demonstrated that the C-S-C- group was more effective in
preventing nephrotoxicity in rats (Jones et al., 1989).
However, not all of the compounds possessing the C-S-C-
group were found to be effective cisplatin antagonists.

The foregoing discussion demonstrates that it is not
possible to predict reliably which particular sulfur-
containing nucleophile will exhibit a platinum-containing
compound protective effect in any particular type of cell,
tissue, or organ. Indeed, individual compounds seem to
exert their protective effects only in certain tissues.
Thus, the ability of any particular nucleophilic sulfur
compound to act as a protective agent in any particular
tissue can only be determined by direct experimentation. Of
course, such compound will only be of value if it does not
substantially reduce the anti-tumor efficacy of cisplatin or
related anti-tumor platinum-containing compounds.

Deegan et al. (1994) demonstrated that male Wistar rats
receiving a single intraperitoneal dose of cisplatin-
methionine at a 1:5 ratio by weight did not exhibit
cisplatin-induced nephrotoxicity. Their results indicated
that cisplatin-methionine is significantly cytotoxic, yet
lacks cisplatin-associated renal toxicity. These workers
suggested a role for either methionine co-treatment or
cisplatin-methionine compounds in the treatment of human
cancers. However, they neither disclosed nor suggested the
specific otoprotective, weight loss-protective,
gastrointestinal-protective, neuroprotective, alopecia-
protective, or survival-enhancing effects of D-methionine
surprisingly discovered by the present inventor. Nor did

they provide any motivation to investigate D-methionine as
an otoprotectant, weight loss-protectant, survival-enhancing
agent, etc., or any reasonable expectation that methionine
could act in these manners during cisplatin administration.

5       Finally, Deegan et al. provided no guidance or suggestion as
to how methionine could be used as a protective agent for
various toxicities in humans, as described herein.  As noted
by Schweitzer (1993; page 12), while various nucleophilic
sulfur protective agents have been shown to be effective in

10      blocking or reversing the renal toxicity of CDDP while
retaining the chemotherapeutic activity of the drug, each
agent has to be considered individually.  The effects on
antineoplastic activity, individual CDDP toxicities, and
appropriate dosing schedules need to be determined on a *per

15      se* basis for each compound.

        In view of the foregoing, the utility of D-Met as a
highly effective platinum-containing anti-neoplastic agent
otoprotectant, weight loss protectant, gastrointestinal
protectant, neuroprotectant, alopecia protectant, and

20      survival-enhancing agent which does not interfere with anti-
tumor activity, and which does not appear to cause any
serious side effects, could not have been predicted.  In
fact, the discovery of D-Met's beneficial effects is
surprising in view of the many significant problems,

25      discussed above, encountered with previously described
sulfur-containing nucleophiles that preclude their clinical
use.


## Summary of the Invention

        The present inventor has addressed the long-felt need
30      in the art for protective agents effective in preventing or
ameliorating various toxic effects of cisplatin and other
platinum-containing anti-tumor compounds, but which do not
significantly affect the antineoplastic activity of these

compounds, and which do not themselves cause deleterious
side effects as a result of their administration.  She has
also addressed the long-felt need in the art for protective
agents effective in preventing or ameliorating various toxic
effects of aminoglycoside antibiotics, loop diuretics, iron
chelating agents, quinine, quinidine, noise, and radiation.
She has surprisingly discovered that D-methionine, and
structurally related compounds, can be used as an
otoprotectant, a weight loss protectant, a gastrointestinal
protectant, a neuroprotectant, an alopecia protectant, and a
survival-enhancing agent during treatment of a mammal with
such compounds, or due to exposure to noise or radiation.

     Accordingly, in one aspect, the present invention
provides a method for preventing or reducing ototoxicity in
a patient undergoing treatment with an aminoglycoside
antibiotic, comprising administering to the patient an anti-
ototoxic effective amount of a methionine protective agent.

     In another aspect, the present invention provides a
method for preventing or reducing ototoxicity in a patient
undergoing treatment with a loop diuretic agent, comprising
administering to the patient an anti-ototoxic effective
amount of a methionine protective agent.

     In a further aspect, the present invention provides a
method for preventing or reducing ototoxicity in a patient
undergoing treatment with an iron chelating agent,
comprising administering to the patient an anti-ototoxic
effective amount of a methionine protective agent.

     In yet a further aspect, the present invention provides
a method for preventing or reducing ototoxicity in a patient
undergoing treatment with quinine or quinidine for
conditions in which such compounds are indicated, comprising
administering to the patient an anti-ototoxic effective
amount of a methionine protective agent.

In another aspect, the present invention provides a
method for preventing or reducing ototoxicity in a patient
exposed to noise for a time and at an intensity sufficient
to result in ototoxicity, comprising administering to the
patient an anti-ototoxic effective amount of a methionine
protective agent.

In yet another aspect, the present invention provides a
method for preventing or reducing ototoxicity,
neurotoxicity, alopecia, gastrointestinal disorder, or
reduced survival in a patient exposed to radiation for a
time and at an intensity sufficient to result in
ototoxicity, neurotoxicity, alopecia, gastrointestinal
disorder, or reduced survival, comprising administering to
the patient an effective amount of a methionine protective
agent.

The present invention also provides compositions,
including pharmaceutical compositions, comprising the
present methionine protective agents, either alone, or in
combination with an aminoglycoside antibiotic, a loop
diuretic agent, an iron chelating agent, quinine, or
quinidine.

Such protective agents can be administered prior to,
simultaneously with, or subsequently to administration of
the aminoglycoside antibiotic, loop diuretic agent, iron
chelating agent, quinine, quinidine, or exposure to a toxic
level of noise or radiation.  Combinations of these time
periods can also be employed.

Further scope of the applicability of the present
invention will become apparent from the detailed description
and drawings provided below.  However, it should be
understood that the following detailed description and
examples, while indicating preferred embodiments of the
invention, are given by way of illustration only since
various changes and modifications within the spirit and

scope of the invention will become apparent to those skilled
in the art from this detailed description.


## Brief Description of the Drawings

The above and other objects, features, and advantages
of the present invention will be better understood from the
following detailed description taken in conjunction with the
accompanying drawings, all of which are given by way of
illustration only, and are not limitative of the present
invention, in which:

Figure 1 shows ABR post-test thresholds (means $\pm$ 1
S.D.) for the various animal groups for all stimuli
including: a) clicks; b) 1000 Hz tonebursts; c) 4000 Hz
tonebursts; d) 8000 Hz tonebursts; and e) 14000 Hz
tonebursts. * indicates significantly different from the
CDDP-treated controls at the $p \leq .01$ level.

Figures 2A-2F are SEM photomicrographs of: A) middle
turn of untreated control; B) middle turn of treated control
(16 mg/kg CDDP); C) middle turn of animal administered 300
mg/kg D-Met prior to the 16 mg/kg CDDP dose; D) basal turn
of untreated control; E) basal turn of treated control (16
mg/kg CDDP); and F) basal turn of animal administered 300
mg/kg D-Met prior to the 16 mg/kg CDDP dose.

Figure 3 shows the average weight loss in grams for the
various animal groups. * indicates significantly different
from the CDDP-treated controls at the $p \leq .01$ level.


## Detailed Description of the Invention

The following detailed description is provided to aid
those skilled in the art in practicing the present
invention. Even so, this detailed description should not be
construed to unduly limit the present invention as
modifications and variations in the embodiments discussed
herein can be made by those of ordinary skill in the art

without departing from the spirit or scope of the present inventive discovery.

The contents of each of the references cited herein are herein incorporated by reference in their entirety.

## Example 1

### Otoprotective Effect of D-Met

This experiment demonstrates the effectiveness of D-Met in preventing a variety of different toxic side effects associated with the use of platinum-containing anti-tumor compounds, exemplified by CDDP (cisplatin), in a mammal.

## Materials and Methods

### Animals

As is well known to those of ordinary skill in the art, the rat is a well-accepted experimental animal useful as a model for studies of CDDP toxicity in humans.

Complete data sets were obtained for five groups of five male Wistar rats (280-421g).  All animals were anesthetized with 1 ml/mg IM of Rompun cocktail (a solution containing 86.21 mg/ml ketamine and 2.76 mg/ml xylazine) prior to all injections and testing.  Anesthesia was supplemented as needed with half doses throughout testing. The five groups included: a treated control group which received 16 mg/kg CDDP dissolved in normal sterile saline (1 mg of CDDP/ml normal saline; solution pH 6.3) administered by i.p. infusion with a Harvard Apparatus Infusion Pump, over a 30 minute period, an untreated control group that received an equivalent volume of normal saline (pH 6.5) instead of CDDP, and three experimental groups that received either 75, 150, or 300 mg/kg D-Met dissolved in 3-5 ml of normal saline (solution pH 6.6) delivered by slow (over 1-2 minutes) i.p. injection 30 minutes prior to the same CDDP infusion as the treated control group.  Both CDDP (purchased

from Sigma Chemical Co., St. Louis) and D-Met (purchased
from Acros Organics, Pittsburgh, PA) were freshly prepared
before each experiment.  For the treated control group, a
total of 10 animals were needed to obtain 5 animals with
complete data sets because 50% of the animals did not
survive to the end of the study period.  Only 5 animals were
needed in the untreated control and in each of the D-Met
pretreated groups because all of the animals in each of
those groups survived until the end of the study period.

All of the care and use of the animals was approved by
the Southern Illinois University School of Medicine
Laboratory Animal Care and Use Committee, and was under the
supervision of the Southern Illinois University School of
Medicine Unit for Laboratory Animal Medicine.

**Evoked Potentials**

Auditory Brainstem Testing (ABR) was used to assess
auditory threshold.  Testing occurred just prior to
administration of the CDDP or saline (with or without a
protective agent) and again 3 days later.  All testing was
performed with the animal in a double walled IAC booth.

Platinum/iridium needle electrodes were placed at the
vertex (non-inverting) to a point directly below the
ipsilateral pinna (inverting) with a ground electrode placed
in the hind leg.

ABR data collection was obtained with a Biologic
Traveler system with an additional custom made high
frequency stimulator for 14000 Hz.  ABR thresholds were
measured in response to 100 microsecond clicks and for
tonebursts with 1 ms rise/fall and 0 ms plateau gated by a
Blackman envelope and centered at the frequencies of 1, 4,
8, and 14 kHz presented at 10/s.  An intensity series was
obtained for each animal from 100 to 0 dB peak equivalent
SPL (peSPL) for click stimuli and Sound Pressure Level (SPL)

for tonebursts in 10 dB decrements.  The term peSPL means
that the amplitude of the click stimulus from the
prestimulus baseline to the first peak is equivalent to the
SPL of a pure tone stimulus having the same prestimulus
baseline to peak amplitude.  Threshold was defined as the
lowest intensity capable of eliciting a replicable, visually
detectable response.

A total of 512 sweeps constituted each average.  The
recording epoch was 15 ms following stimulus onset.
Responses were analogue filtered with a 30-3000 Hz bandpass.

Rectal temperature was monitored throughout recordings,
with animal temperature being maintained by a warming pad.

**Electron microscopy**

The animals were sacrificed by decapitation while under
general anesthesia and cochleae perfused with fixative
through the perilymphatic spaces.  The primary fixative was
2.5% glutaraldehyde at 4°C in 0.1M phosphate buffer (pH
7.4).  A small hole in the otic capsule was hand drilled
beneath the first turn with a three sided, sharpened pick.
In vitro perfusion was performed intermittently within 5
minutes of sacrifice through the small hole in scala
tympani, allowing the fluid to exit through the opened oval
window.  After perfusion fixation, the round window membrane
was removed, and the cochleae were immersed in
glutaraldehyde and stored in the refrigerator overnight.

After overnight fixation in glutaraldehyde, the
cochleae were rinsed in 0.1 M phosphate buffer and gently
perfused with the buffer through the perilymphatic spaces by
loosely fitting the tube end of the perfusion syringe over
the opening drilled in the scala tympani.  Cochleae were
then rinsed in buffer 3 times.  After rinsing, the cochleae
were post-fixed by a perfusion of 1.5% $OsO_4$ (at 4°C) in
phosphate buffer in a fume hood.  Fixation was continued by

immersion and rotation in the same fixative for 15 minutes.
The cochleae were rinsed in the same fashion as after
glutaraldehyde fixation.

Under the dissecting microscope, the bony capsule of
the cochlea was carefully removed.

The tissue was then serially dehydrated in 2 X 50%,
70%, 85%, 95% and 3 X 100% ethanol.  Each specimen was dried
using Peldri and placed on a stub for sputter coating with
13 nm platinum.  The tissue was viewed through a Hitachi S-
500 scanning electron microscope and photographs taken on
Polaroid type 55 land Film.

Semi-quantitative analysis per turn for the outer hair
cells was performed in the following manner:  For each turn
of the cochlea, apical, middle, and base, a representative
sample was examined.  For each sample, 11 inner hair cells
served as a guide to count a section of 33 outer hair cells
or 11 per row.  The number of damaged or missing outer hair
cells within each sample was then counted.

**Weight**

Each animal's weight was measured in an Ohaus triple
beam balance scale before administration of the anesthetic
for the pretest and again before the post-test 3 days later.

**Statistical Analysis**

ABR data were analyzed using a three factor analysis of
variance (ANOVA) with one between subject factor (groups)
and two within subject factors (frequency and pre- vs. post-
test).  Each dependent variable was analyzed independently.
Tests subsequent to the ANOVA were carried out in accordance
with the Tukey HSD procedure.  Weight loss and/or
gastrointestinal protection was measured using the same type
of statistical analysis as the ABR measures.  SEM data were
analyzed for each turn using a one way analysis of variance

with Post-Hoc Tukey HSD analysis.  The criterion for statistical significance for all measures was p≤0.01.

## Results

### Hearing loss

5          Post test ABR hearing thresholds are presented in Figure 1.  As expected, no significant threshold shift in response to any stimulus occurred in the untreated control group, and marked significant threshold shift occurred in response to all stimuli, but particularly for the high frequencies, in the treated control group.  For the animals receiving D-Met prior to the CDDP, 2/5 and 3/5 animals receiving 75 and 150 mg/kg D-Met, respectively, had complete otoprotection as defined by no significant ABR threshold shift for any stimulus.  For the 300 mg/kg D-Met
15     administration, all 5 animals had complete otoprotection for all stimulus conditions (Fig. 1).  All experimental groups receiving any level of D-Met had significantly lower ABR thresholds than the treated control group for all stimuli, as did the untreated control group.  This observed
20     protection from hearing loss may occur not only as a result of protection of cochlear mechanisms, but also as a result of protection of the auditory neural pathway (i.e., neuroprotection).

25     ### Histology

          Histologic findings (Fig. 2) were consistent with the ABR findings.  All groups had essentially normal hair cell counts for the apical turn, with no significant difference between groups.  For the middle and basal turns, only the
30     treated control group showed significantly different findings from the untreated control group and from the three groups receiving preadministration of D-Met, with the basal turn being consistently more affected than the middle turn.

## Weight loss

CDDP-induced weight loss diminished as D-Met dosing increased (Fig. 3). Weight loss in the experimental group receiving 300 mg/kg was significantly less than that in the treated control group. The amount of weight loss across groups was significantly correlated with the amount of threshold shift for all stimuli, with the highest correlation for the 14 kHz stimulus.

## Neuroprotection

Animals receiving D-Met were noticeably more lively, active, and coordinated on the morning of the third day as compared to the surviving treated control group animals.

## Alopecia

The coats of animals receiving D-Met were noticeably superior to those of control group animals, and showed significantly less hair loss.

## Survival during the study period

All 15/15 animals receiving any level of D-Met survived to the end of the study period as compared to 5/10 treated control group animals.

## Discussion

The foregoing results demonstrate that 300 mg/kg D-Met administered 30 minutes before 16 mg/kg CDDP provides complete otoprotection, as indicated by ABR and histologic findings, while also reducing CDDP-induced weight loss, gastrointestinal toxicity, neurotoxicity, alopecia, and improving survival.

While not intending to be bound to any particular theory, I hypothesize that D-Met may provide these

protective effects by any one or more of a number of
different mechanisms.

According to Schweitzer, (1993), sulfur-containing
compounds may prevent CDDP from interacting with
intracellular target molecules, the nucleophilic oxygen or
sulfur atoms interacting with the electrophilic site of the
CDDP, thus displacing or extracting platinum after it is
bound.  Theoretically, these agents provide protection
because of their high affinity for platinum complexes.  It
is known that CDDP reacts with methionine's sulfhydryl group
(Lempers and Reedijk, 1990).

CDDP may preferentially bind to free D-Met, thus
protecting glutathione.  Reduced glutathione is an essential
part of the anti-oxidant pathways.  CDDP does reduce renal
glutathione levels, resulting in increased lipid
peroxidation (Hanneman and Baumann, 1988; Sugihara et al.,
1987a, b; Boogaard, 1991).  CDDP also reduces glutathione
levels in the cochlea and inferior colliculus (Ravi et al.,
1991).  More recent work (Ravi et al., 1995, Rybak et al.,
1995) investigated changes specifically in the cochlear
antioxidant system.  Systemic CDDP administration decreased
reduced glutathione (GSH) levels, and reduced activity of
the enzymes glutathione peroxidase (GSH-Px) and glutathione
reductase (GR).  Oxidized glutathione or glutathione
disulfide (GSSG) was not found, suggesting that the overall
glutathione levels decreased rather than merely being
oxidized.  Ravi et al., (1995) also reported increased
cochlear malondialdehyde (MDA) levels, reflecting increased
lipid peroxidation.  Because CDDP does increase the level of
free radicals in general (Hanneman and Baumann, 1988),
preservation of the anti-oxidant system may be critical in
preventing CDDP side effects.

D-Met preadministration may protect the sulfur groups
of proteins, including protein bound L-methionine.  CDDP

binds to the methionine groups in protein and to glutathione
(Lempers and Reedijk, 1990).  Schweitzer, (1993) suggests
that platinum binding to protein sulfhydryl groups may cause
CDDP nephrotoxicity, accounting for the nephroprotective

5      action of thiols (Gandara et al., 1989).  It is logical that
free D-Met may preferentially bind to CDDP because of the
steric hindrance of the protein bound sulfur groups.  This
protection could occur by preferential binding of the CDDP
to D-Met, or perhaps D-Met could reverse the Pt binding to

10     the protein-bound methionine and glutathione, as do other
sulfur-containing compounds (Lempers and Reedijk, 1990).
Methionine can displace plasma-bound Pt (Alden and Repta,
1984).

       D-Met binding to CDDP may also protect free
15     L-methionine (L-Met), an essential amino acid.  Parenteral
administration of DL-methionine in humans results in higher
plasma levels of the D- isomer (Printen et al., 1979).
Because the D-Met is less well metabolized than L-Met in
humans, it may remain more available for CDDP binding, thus

20     protecting the L-Met for needed protein synthesis, cell
activation, and metabolism.

       Fortunately, D-Met does not inhibit CDDP anti-tumor
action as determined against the Walker 256 carcinosarcoma
in the rat (Jones and Basinger, 1989).  Preadministration of

25     methionine, presumably a racemic mixture, actually
sensitized NHIK 3025 *in vitro* human uterine cervix carcinoma
*in situ* cancer cells to CDDP cytotoxicity (Melvik and
Petterson, 1987).

       Several factors may account for D-Met's CDDP-
30     protective action in nontumor cells as compared to tumor
cells.  Methionine metabolism is clearly different in tumor
and nontumor cells (Hoffman, 1985), but how these
differences may result in differential CDDP action has not
been elucidated.  The toxic effects of CDDP may also be

different in tumor and nontumor cells.   The CDDP anti-tumor
effect results primarily from cisplatin's reaction with DNA,
primarily at the N-7 bisguanine position.   Initially, mono-
adducts are formed, followed by rapid intra-strand cross-
linking, causing cytotoxicity (see the review by Tognella,
1990).   The binding of platinum to cytosolic ligands and
nucleoprotein fractions may also play a role, but the
receptors and interactions are not yet defined (Schweitzer,
1993).   Significant DNA binding in normal cells is less
likely because fewer DNA replication forks are open at any
point in time, unlike in rapidly dividing tumor cells.   In
nontumor cells, the toxic effects may be largely secondary
to the binding with amino acids, either free or protein-
bound, and deactivation of the antioxidant pathway, as
described above.

     The timing of CDDP reactions may also be different in
tumor and nontumor cells.   CDDP uptake by the Walker 256
carcinosarcoma in the rat is very rapid, occurring in the
first few minutes after administration, followed by a rapid
redistribution that is complete within 15 minutes after
injection (Jones and Basinger, 1989).   Because the uptake of
CDDP into tumor cells is very rapid, the binding to the DNA
bisguanine groups, particularly at the open replication
forks, may occur more rapidly than the reaction of CDDP with
methionine.

     Although CDDP uptake into the kidney is also rapid
(Jones and Basinger, 1989), CDDP binding to protein is
relatively slow.   As reviewed by Schweitzer, (1993),
following IV cisplatin administration, 90% of cisplatin is
protein-bound within 2 hours, with half-lives of 25 to 50
minutes and 53 to 73 hours for unbound and bound platinum,
respectively.   Platinum tissue levels decline slowly.
Platinum may still be measured over a week after high dosage
administration, and bound fragments may still be present

when the patient starts the next treatment cycle.  Platinum
uptake in the stria vascularis and the organ of Corti
increases at least over a 24 hour period, which may underlie
the dose-related cumulative ototoxicity (Schweitzer, 1993),
but may also allow time for CDDP binding to D-Met before
uptake into the cochlea.

However, the CDDP toxicities both in tumor and nontumor
cells are complex, and many factors may be involved in D-
Met's protective action.

A positive correlation between weight loss and outer
hair cell loss in guinea pigs has been demonstrated (Tange
et al., 1982, Hoeve et al., 1988), but both studies noted
marked intersubject variability.  The data presented above
reveal a positive correlation between weight loss and
threshold loss that increased as stimulus frequency
increased.  The significant reduction in weight loss with
300 mg/kg D-Met preadministration suggests that D-Met also
alleviates some of the gastrointestinal toxicities of CDDP.
The amelioration in weight loss by D-Met could also be
related to a decrease in nephrotoxicity or other factors.

The elimination of CDDP mortality in this study by
preadminstration of any of the three D-Met levels
demonstrates a marked improvement in the overall health
status of the animals.  D-Met preadministration may
therefore be useful in shifting the $LD_{50}$ level of CDDP and
other platinum-containing anti-tumor agents, permitting the
safe use of higher levels of these agents during
chemotherapy, with potential improvement of the cancer cure
rate.


## Therapeutic Applications

The data presented above demonstrate that D-Met
prevents CDDP-induced ototoxicity, reduces CDDP-induced
weight loss, protects against CDDP-induced gastrointestinal

toxicity, neurotoxicity, and alopecia, and improves survival
during CDDP treatment in a mammal.  Because CDDP-induced
hearing loss in humans is almost invariably permanent,
prevention of this hearing loss has a number of important
consequences.  If ototoxicity could be prevented, not only
could hearing be spared, but perhaps higher doses of
cisplatin and other platinum-containing antineoplastic
agents could be routinely employed during chemotherapy,
increasing the effectiveness of anti-tumor therapy in human
patients.

        The foregoing data also suggest that D-Met and the
other methionine protective agents disclosed herein will be
effective in the treatment of noise-induced, loop diuretic-
induced, aminoglycoside antibiotic-induced, iron chelator-
induced, quinine- and quinidine-induced, and radiation-
induced ototoxicity, as well as in ameliorating other
radiation-induced side effects such as neural damage,
alopecia, gastrointestinal disorders, and in improving
patient survival.

        As used herein, the term "ototoxicity" includes, but is
not limited to, any detrimental or pathologic change in the
structure or function of the ear, including changes in
hearing and balance.  Auditory functional changes can
include, but are not limited to, hearing loss or other
changes in auditory threshold for any stimulus, perception
of sound including recruitment (abnormal growth in the
perception of loudness), ability to identify, localize,
recognize, distinguish between, or process sounds, and/or
distortion of sounds or any abnormality as measured by
conventional auditory tests.  This term also includes
tinnitus (ringing or noises in the ear), which includes any
perception of sound that is not in response to an external
signal.  Further, ototoxicity includes any perceived or
measured functional change in the balance or vestibular

system, including, but not limited to, either induced or spontaneous vertigo, dysequilibrium, increased susceptibility to motion sickness, nausea, vomiting, nystagmus, syncope, lightheadedness, dizziness, difficulty in visual tracking secondary to vestibular or balance disorder or abnormality as measured on any test of vestibular or balance function.  Structural changes can include any intra- or extra-cellular, multicellular, or organ change in the auditory or vestibular pathways from the external ear up through and including the cortex and all pathways in between.

The term "otoprotective agent" refers to an agent that prevents, ameliorates, or otherwise protects against ototoxicity.

The term "neurotoxicity" includes, but is not limited to, any detrimental or pathologic change in the structure or function in the neurologic system or any part thereof. Neurologic functional changes can include, but are not limited to, neuropathy, either central or distal, including a common "stocking and glove" pattern, tingling, loss of sensation, numbness, decreased vibratory sensation, decreased deep tendon reflexes, sensory ataxia, neuritis, focal encephalopathy, aphasia, autonomic neuropathy, postural hypotension, a myasthenia-like syndrome, muscle cramps, headache, seizures, blindness or visual disturbance secondary to disorder of the optic or visual neurological pathway, papilledema, hearing loss secondary to disorder of the auditory neurologic pathway, and/or loss of the sensation of taste.  Structural changes can include intra- or extra-cellular, multicellular, or organ changes, anywhere in the neurologic system, including both peripheral and central systems.  Neurotoxicity can manifest itself during or after the course of treatment with platinum-containing anti-tumor compounds.

The term "neuroprotective agent" refers to an agent that prevents, ameliorates, or otherwise protects against neurotoxicity.

The term "gastrointestinal toxicity" includes, but is not limited to, any detrimental or pathologic change in the structure or function in the gastrointestinal system or any part thereof.  Gastrointestinal changes include, for example, current or delayed nausea, vomiting, esophageal reflux, stomatitis, bleeding along the gastrointestinal tract, diarrhea, weight loss, and/or anorexia. Gastrointestinal toxicity can manifest itself during or after the course of treatment with platinum-containing anti-tumor compounds.

The term "gastrointestinal-protective agent" refers to an agent that prevents, ameliorates, or otherwise protects against gastrointestinal toxicity.

In view of the results presented above, the medical or veterinary practitioner, by employing the compounds, compositions, and methods described below, will be able to maintain any of the foregoing parameters in a mammal, especially a human, at a level of from about 70% to about 80% of the pre-chemotherapy or other treatment or exposure level, more preferably from about 80% to about 90% of the pre-chemotherapy or other treatment or exposure level, most preferably from about 90% to about 100% of the pre-chemotherapy or other treatment or exposure level, as measured by standard tests routinely employed in the art. These compounds and methods can also be used for the treatment of domestic pets, such as cats and dogs.

The teachings presented herein permit the design of therapeutic regimens that can be employed to reduce the undesirable side effects of platinum-containing anti-tumor compounds such as CDDP, increase the dosing of such anti-tumor compounds to obtain a higher cancer cure rate, and

perhaps include weaker patients in treatment protocols employing such anti-tumor compounds, from which they are currently excluded because they cannot withstand the toxicities associated therewith.  The presently disclosed teachings also permit the design of therapeutic regimens useful in preventing or reducing the undesirable ototoxic side effects of noise, loop diuretics, aminoglycoside antibiotics, iron chelators, quinine, quinidine, and radiation, as well as other radiation-induced side effects such as neural damage, alopecia, gastrointestinal disorders, and decreased patient survival.

Administration of D-Met before, during, or after administration of antineoplastic effective amounts of platinum-containing anti-tumor compounds such as CDDP, or during various combinations of these time periods, is particularly useful in view of D-Met's lack of interference with CDDP anti-tumor action (Jones and Basinger, 1989; Melvik and Petterson, 1987).
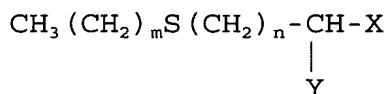
D-Met and structurally related compounds can be used in conjunction with platinum-containing antitumor compounds such as CDDP during chemotherapy, and in conjunction with the use of loop diuretics, aminoglycoside antibiotics, iron chelators, quinine, and quinidine, as described below. These methionine protective agents can also be used to prevent or reduce the ototoxic effects of noise and radiation, as well as other radiation side effects, as described below as well.

**Methionine and Its Derivatives**

D-Met has been administered to humans for various purposes.  For example, C-labeled D-Met has been used for radiographic imaging (Meyer et al., 1985), and DL-methionine has been administered for parenteral nutrition (Printen et al., 1979).  D-Met has also been safely administered to

humans orally for nutritional studies (Kaji et al., 1987;
Kies et al., 1975; Stegink et al., 1986).  Oral methionine
is sold as an over the counter preparation to control
urinary pH (Drug Facts and Comparisons, 1991).  The
contraindications are for patients with a history of liver
disease, and that high dosage methionine may inhibit growth
in children when given for an extended time period.

Analogs or derivatives of methionine useful in the
present invention are compounds containing a methionine
moiety, or a methionine-like moiety including a thioether
group, that exhibit an otoprotectant effect, a weight-loss
protectant effect, a gastrointestinal protectant effect, a
neuroprotectant effect, an alopecia protectant effect,
and/or a survival-enhancing effect when used in conjunction
with an antitumor platinum coordination compound
administered in an effective chemotherapeutic dose, a loop
diuretic compound, an aminoglycoside antibiotic, an iron
chelator, quinine, or in conjunction with exposure to noise
or radiation.  Among the compounds structurally related to
D-Met that can be employed in the present invention are
those containing the C-S-C- (thioether) moiety.  These
include, but are not limited to, compounds having the
structural formula:

$$CH_3(CH_2)_mS(CH_2)_n-\underset{\underset{Y}{|}}{CH}-X$$

wherein m is an integer from 0 to 3; n is an integer from 1
to 3; X = $-OR^1$, $-OCOR^1$, $-COOR^1$, $-CHO$, $-CH(OR^1)_2$, or
$-CH_2OH$; Y = $-NR^2R^3$ or $-OH$; $R^1$ = H or a substituted or
unsubstituted, straight, branched chain, or cyclic alkyl
group having 1 to 6 carbon atoms, preferably 1 to 4 carbon
atoms; $R^2$ = H or a substituted or unsubstituted, straight or

branched chain acyl group having 1 to 6 carbon atoms,
preferably 1 to 4 carbon atoms; and $R^3$ = H or a substituted
or unsubstituted, straight or branched chain acyl group
having 1 to 6 carbon atoms, preferably 1 to 4 carbon atoms;

5       or a pharmaceutically acceptable salt thereof.

        The lower alkyl and acyl groups described herein,
either alone or containing the various substituents defined
herein, can contain from one to six carbon atoms in the
principal chain, and up to about 15 carbon atoms total.  The

10      lower alkyl groups include, for example, methyl, ethyl,
propyl, isopropyl, butyl, hexyl, cyclopropyl, cyclopentyl,
cyclohexyl, and the like.  Substituents of the substituted
alkyl and acyl groups described herein can include, for
example, groups selected from alkyl, cycloalkyl, alkenyl,

15      alkynyl, aryl, heteroaryl, O, S, N, P, or halogen (Cl, F,
Br, or I) atoms.  Optionally, these substituent alkyl,
cycloalkyl, etc., groups can be substituted with O, S, N, P,
or halogen (Cl, F, Br, or I) atoms.  These substituent
alkyl, cycloalkyl, etc., groups include, for example, lower

20      alkoxy groups such as methoxy, ethoxy, and butoxy, and
groups such as halo, nitro, amino, and keto.

        The alkenyl groups described herein, either alone or
with the various substituents defined herein, are preferably
lower alkenyl containing from two to six carbon atoms in the

25      principal chain, and up to about 15 carbon atoms total.
They can be substituted, straight, or branched chain, and
include ethenyl, propenyl, isopropenyl, butenyl, isobutenyl,
hexenyl, and the like.

        The alkynyl groups described herein, either alone or

30      with the various substituents defined herein, are preferably
lower alkynyl containing from two to six carbon atoms in the
principal chain, and up to about 15 carbon atoms total.
They can be substituted, straight or branched chain, and

include ethynyl, propynyl, butynyl, isobutynyl, hexynyl, and
the like.

The aryl moieties described herein, either alone or
with various substituents defined herein, can contain from
about 6 to about 15 carbon atoms, and include phenyl.
Substituents include alkanoxy, protected hydroxy, halogen,
alkyl, aryl, alkenyl, acyl, acyloxy, nitro, amino, amido,
etc.  Phenyl is a preferred aryl.

The heteroaryl moieties described herein, either alone
or with various substituents defined herein, can contain
from about 5 to about 15 atoms, and include, furyl, thienyl,
pyridyl and the like.  Substituents include alkanoxy,
protected hydroxy, halogen, alkyl, aryl, alkenyl, acyl,
acyloxy, nitro, amino, and amido.

The acyloxy groups described herein can contain alkyl,
cycloalkyl, alkenyl, alkynyl, aryl, or heteroaryl groups.

The carbon atoms, i.e., the methyl and methylene
groups, constituting the principal backbone of the
methionine or methionine-like moiety can also be substituted
as variously described above.

Non-limiting examples of such methionine protective
agents include D-methionine (D-Met), L-methionine, a mixture
of D-methionine and L-methionine, normethionine,
homomethionine, methioninol, hydroxy methionine, ethionine,
or pharmaceutically acceptable salts thereof.  S-adenosyl-L-
methionine, or a pharmaceutically acceptable salt thereof,
can also be employed.  Methionine protective agents of the
present invention can be in the D-, L-, or DL- form, and
include pharmaceutically acceptable N-(mono- and
dicarboxylic acid) acyl derivatives and alkyl esters
thereof.  Exemplary acyl derivatives include the formyl,
acetyl, propionyl, and succinyl derivatives.  Exemplary
ester derivatives include the methyl, ethyl, propyl,
isopropyl, and butyl esters.  D-Met is a preferred compound.

Collectively, methionine, along with the other
compounds discussed above, can be referred to as "methionine
protective agents."  These compounds can be used alone or in
various combinations with one another in the methods
5          described herein.

These compounds can be administered alone, or in
combination with the other drug compounds discussed herein,
in the form of the water-soluble acid, free base, or as
physiologically acceptable salts, including acid addition
10         salts formed with organic and inorganic acids, for example,
hydrochlorides, hydrobromides, sulfates, phosphates,
citrates, fumarates, and maleates, and cations such as
sodium, potassium, etc.  These compounds can be formulated
for administration to humans and animals with
15         pharmaceutically acceptable carriers, excipients, and
diluents, such as sterile distilled water, Ringer's
solution, normal saline, 5% glucose, dextrose, fructose,
sucrose, etc., and mixtures thereof, as is well known in the
art.  Antimicrobial agents, preservatives, etc., can also be
20         included.  Compositions for oral administration can include
coloring and flavoring agents.  Additional methods of
formulating compounds of the present invention for
administration in the methods described herein can be found,
for example, in *Remington's Pharmaceutical Sciences*,
25         Fifteenth Edition, Mack Publishing Company, Easton,
Pennsylvania, 1975.


## Anti-tumor Platinum Compounds

Cisplatin (CDDP; *cis*-diamminedichloro-platinum(II)) is
30         currently the anti-tumor platinum coordination compound most
frequently employed in the therapy of testicular cancer,
ovarian tumors, and a variety of other cancers.  Methods of
employing CDDP clinically are well known in the art
(Nicolini, 1987).  For example, CDDP can be administered in

a single day over a six hour period, once per month, by slow
intravenous infusion.  For localized lesions, CDDP can be
administered by local injection.  Intraperitoneal infusion
can also be employed.  CDDP can be administered in doses as
low as 10 mg/m$^2$ per treatment if part of a multi-drug
regimen, or if the patient has an adverse reaction to higher
dosing.  At the low end, a more common clinical dose is
about 30 mg/m$^2$; the high end of the range is about 120 to
about 150 mg/m$^2$ per treatment.  When used in conjunction with
D-Met or other methionine protective agents, these dosages
can be increased.

CDDP is representative of a broad class of water-
soluble, platinum coordination compounds well known in the
art that provide platinum in the form of an ion having anti-
tumor activity.  Among the anti-tumor platinum coordination
compounds described in the literature which are useful in
the methods of the present invention are, for example,
*trans*-diaminedichloro-platinum(II), *cis*-diamine-
diaquaplatinum(II)-ion, *cis*-diaminedichloroplatinum(II)-ion,
chloro(diethylenetriamine)-platinum(II) chloride,
dichloro(ethylenediamine)-platinum(II), diammine(1,1-
cyclobutanedicarboxylato)-platinum(II) (carboplatin),
spiroplatin, dichlorotrans-dihydroxybisisopropolamine
platinum IV (iproplatin), diammine(2-ethylmalonato)-
platinum(II), ethylenediamine-malonatoplatinum(II),
aqua(1,2-diaminodiclohexane)-sulfatoplatinum(II), (1,2-
diaminocyclohexane)malonato-platinum(II), (4-
carboxyphthalato)(1,2-diaminocyclo-hexane)-platinum(II),
(1,2-diaminocyclohexane)-(isocitrato)platinum(II), (1,2-
diaminocyclohexane)-*cis*(pyruvato)platinum(II), and (1,2-
diaminocyclohexane)-oxalatoplatinum(II).


**Loop Diuretics**

Loop diuretics are a group of compounds with dissimilar chemical structure, but which share a similar mechanism and site of action within the kidney: these compounds inhibit sodium chloride reabsorption at the high-capacity site in the thick ascending limb of the loop of Henle, causing greatly increased excretion of sodium chloride in the urine, and to a lesser extent of potassium.  Loop diuretics are among the compounds exhibiting the greatest diuretic effect, and are commonly used in the treatment of edema of cardiac, hepatic, or renal origin.  Use of these compounds can cause ototoxicity at least in part as a result of the alteration of electrolyte composition in the inner ear.  In adults, ototoxicity is generally reversible, disappearing upon withdrawal of the drug; however, permanent hearing loss has been reported, particularly with ethacrynic acid.  In neonates, reversibility of hearing loss may be permanent. Commonly used loop diuretics include, but are not limited to, furosemide (Lasix and other compounds), ethacrynic acid (Edecrin), bumetanide (Bumex and other compounds), piretanide, muzolimine, indapamide (Lozol), and xipamide.

Loop diuretics greatly exacerbate the ototoxicity of platinum-containing antitumor compounds and aminoglycoside antibiotics.  The interaction of these ototoxic compounds is synergistic; D-met and the other methionine protective agents disclosed herein may prevent their ototoxicity or ototoxic synergistic interaction.

**Aminoglycoside Antibiotics**

The aminoglycoside antibiotics share several structural features: they each contain one or more sugar moieties and a streptidine ring, and they each have one or more amino or guanidino groups.  The currently available aminoglycoside antibiotics include streptomycin, kanamycin, gentamicin, amikacin, neomycin, netilmicin, paromomycin, vancomycin,

hygromycin, erythromycin and tobramycin.  One of the
principal dangers associated with the use of aminoglycoside
antibiotics is their ototoxicity, which is associated with
either hearing loss (cochlear damage), vertigo (vestibular
damage), or both.  An early sign is tinnitus accompanied by
loss of high-frequency hearing.  Early detection of hearing
loss can be reversed; prolonged treatment results in
permanent hearing loss.  The concomitant administration of
other drugs that cause similar adverse effects potentiates
the adverse effects of the aminoglycosides.  Such other
drugs include loop diuretic agents, as discussed above.

## Iron Chelating Agents

Iron chelating agents such as deferoxamine mesylate
(desferrioxamine mesylate; for example, Desferal) are used
to treat patients exhibiting elevated levels of iron in the
blood.  Such patients include those suffering from sickle
cell anemia, hereditary disorders resulting in elevated
blood iron levels, those receiving frequent blood
transfusions, those who have ingested large amounts of
ferrous salts of iron (iron poisoning), etc.  The use of
iron chelating agents such as deferoxamine can result in
ototoxicity.

## Quinine and Quinidine

Quinine (Quinamm) has long been used as an antipyretic,
analgesic, and antimalarial.  Recently, it has been used to
stabilize muscle membranes against repetitive activity.
Specifically, it is used to treat myotonia congenita
(Thomsen's disease) and nocturnal muscle cramp.  Quinidine
is a class IA antiarrhythmic agent useful in the treatment
of atrial and ventricular arrhythmias.

The adverse side effects of quinine and quinidine are
similar, and have been given the name "cinchonism," deriving

from the fact that quinine is obtained from the bark of the
cinchona tree.   These side effects include disturbances of
hearing, including tinnitus, deafness, and vertigo.

## Noise

Noise-induced hearing loss, both impulse and chronic
exposure, can damage hearing.   In impulse noise, including
blast exposure, the patient may suffer significant tympanic
membrane and middle ear damage.   In chronic exposure, which
generally occurs at lower intensity levels, middle ear and
tympanic membrane damage are unlikely.   In noise exposure,
the primary and initial damage is generally cochlear, with
secondary neural degeneration of the auditory system
occurring over time.   Noise-induced hearing loss has been
reviewed by the present inventor in the book entitled
*Essential Audiology For Physicians* (1998) Singular
Publishing Group, Inc., San Diego.

Noise-induced hearing loss can occur secondary to a
single very loud noise exposure, or secondary to relatively
high-level noise exposure over a long period of time.   The
risk of noise-induced hearing loss is related to both sound
intensity and duration.   Both the Occupational Safety and
Health Administration (OSHA) and the Environmental
Protection Agency (EPA) have established standards relevant
to noise exposure levels in industry.   The OSHA Permissible
Noise Exposure Levels range from a duration of 32 hours at a
sound level of 80 dBA to 0.25 hours at 115 dBA.   For every 5
dB increase in the noise level, the allowable exposure
duration is halved.   Non-industrial, e.g., recreational,
noises intense enough to damage hearing can vary, for
example, from approximately 90 dBA (lawnmower) to
approximately 140 dBA (shotgun blast).

## Radiation

Exposure to radiation, whether intentional, as in radiation therapy, or unintentional, as by accident, war, etc., can result in ototoxicity, as well as neural damage (neurotoxicity), alopecia, gastrointestinal disorders, and reduced patient survival.  Although physical rather than chemical, radiation can be considered another "ototoxin" in view of its toxicity to the ear and hearing.  Radiation-induced hearing loss is more likely to involve the middle ear than is hearing loss caused by platinum-containing compounds or loop diuretics; however, cochlear and neural problems can also occur.

Radiation-induced ototoxicity, for example hearing loss, can occur as a result of exposure to 35-40 Gy or higher, either as a single or cumulative dose.  Radiation-induced gastrointestinal toxicity, which is similar to that occurring during chemotherapy, includes electrolyte loss, secondary infections, bloody diarrhea, and gastrointestinal bleeding, and can occur upon exposure to a radiation dose in the range from 5-20 Gy, or higher.

## Administration of Methionine Protective Agents

The methionine protective agents of the present invention can be administered orally or parenterally, for example intraperitoneally, by intravenous injection, intravenous infusion, etc., as described in *Remington's Pharmaceutical Sciences*, Fifteenth Edition, Mack Publishing Company, Easton, Pennsylvania, 1975.  These protective agents can also be given by local administration, for example, when the platinum-containing chemotherapeutic agent is administered by local injection, as noted above.  Localized administration of methionine protective agents can be carried out by topical application employing pharmaceutical formulations designed for this purpose as is known in the art, local injection, etc.

In one embodiment of the present invention, a
methionine protective agent is administered topically to the
round window membrane of the ear.  Typically, such topical
administration is carried out by applying a pharmaceutical
formulation such as a topical solution comprising the
otoprotective agent to the round window membrane by a micro-
catheter or by injection into the middle ear.  Suitable
micro-catheters include those commercially available, for
example, from IntraEar Corp., Denver, Co.  Preferably, the
micro-catheter is attached to a battery-operated pump such
as that commercially available from Disetronics, Inc., which
is capable of automatically applying a topical solution
comprising the otoprotective agent to the round window
membrane, either continuously or intermittently.

Administration of the methionine protective agents of
the present invention simultaneously with the administration
of a platinum-containing chemotherapeutic agent, loop
diuretic agent, aminoglycoside antibiotic, iron chelating
agent, or quinine or quinidine, can be accomplished in
several ways.  For example, each agent can be formulated
individually and administered separately at the same time
via any of the routes described herein or which are
otherwise conventional in the art.  Alternatively, both can
be contained together in a single dose formulation that can
be administered by a single route.  As in the case of the
platinum-containing chemotherapeutic agent, loop diuretic
agent, etc., the dose of methionine protective agent can be
administered in a single day.


**Dosages**

The methionine protective agents discussed above can be
employed in methods for treating human and animal patients
undergoing treatment with anti-cancer effective amounts of
platinum-containing chemotherapeutic agents to prevent or

reduce ototoxicity, weight loss, gastrointestinal toxicity, neurotoxicity, alopecia, and to prolong survival.  In addition, these methionine protective agents can be employed in methods for treating human and animal patients undergoing treatment with diuretic-effective amounts of loop diuretic agents, antibiotic-effective amounts of aminoglycoside antibiotics, iron chelating-effective amounts of iron chelating agents, effective amounts of quinine or quinidine, or in methods for treating human and animal patients exposed to ototoxic noise levels, and methods for treating human and animal patients exposed to radiation levels capable of causing ototoxic effects such as hearing loss, as well as radiation-induced neural damage, alopecia, and gastrointestinal disorders.  The present methionine protective agents can also improve survival in patients exposed to radiation.

These methods comprise administering to the patient an appropriate effective amount of a methionine protective agent prior to, simultaneously with, or subsequent to administration of a platinum-containing chemotherapeutic agent, loop diuretic agent, aminoglycoside antibiotic, iron chelating agent, or quinine or quinidine, or exposure of the patient to noise or radiation.  Combinations of these time periods can also be employed.

Typically, the methionine protective agents of the present invention can be administered orally; parenterally by intravenous injection or slow infusion; intraperitoneally; or topically by application to the round window membrane of the ear.  When administered parenterally, the effective amount of methionine protective agent can be in the range of from about 1.0 mg/kg body weight to about 600 mg/kg body weight.  More preferably, the effective amount of methionine protective agent ranges from about 5 mg/kg body weight to about 500 mg/kg body weight, even more

preferably from about 10 mg/kg body weight to about 400 mg/kg body weight.

Alternatively, the effective amount of methionine protective agent can be expressed on a mole:mole basis in relation to the anti-cancer effective amount of platinum-containing chemotherapeutic agent. This effective amount can be in the range of from about 4:1 to about 167:1, more preferably from about 4.25:1 to about 100:1, and most preferably from about 4.68:1 to about 20:1, methionine protective agent:platinum-containing chemotherapeutic agent, on a molar basis. A dosing ratio of about 18.75:1 on a molar basis is a preferred ratio.

If necessary, the amounts and ratios described above can be modified for different platinum-containing chemotherapeutic agents, loop diuretic agents, aminoglycoside antibiotics, iron chelating agents, and quinine or quinidine, or exposure to noise and radiation, by routine optimization, including monitoring of effectiveness and titration for the desired effect, by the methods described herein.

When administered orally, the methionine protective agent should be given in an amount that will result in a blood serum level equivalent to that achieved by the parenterally administered dosages set forth above. Such effective oral dosages can easily be determined by one of ordinary skill in the art via conventional *in vitro* or *in vivo* methods such as those described in *Remington's Pharmaceutical Sciences*, Fifteenth Edition, Mack Publishing Company, Easton, Pennsylvania, 1975.

When administered topically to the round window membrane of the ear, the effective amount of otoprotective agent is typically administered as a pharmaceutical formulation such as a topical solution. Generally, topical administration comprises applying from about 0.001 ml to

about 0.010 ml, preferably from about 0.0025 ml to about
0.0075 ml, and most preferably about 0.005 ml of a topical
solution comprising the otoprotective agent to the round
window membrane.  The topical solution typically comprises
from about 10 mg/ml to about 50 mg/ml, preferably from about
20 mg/ml to about 30 mg/ml, and most preferably about 25
mg/ml of otoprotective agent.

## Treatment Regimen

In the various methods of the present invention, the
effective amount of sulfur-containing protective agent can
be administered prior to, contemporaneously with, or
subsequent to administration of the effective amount of
platinum-containing chemotherapeutic agent, loop diuretic
agent, aminoglycoside antibiotic, iron chelating agent, or
quinine or quinidine, or exposure of the patient to harmful
noise or radiation.  Combinations of these time periods can
also be employed.  Generally, prior administration of the
effective amount of the otoprotective agent can be conducted
broadly within the period ranging from as much as 14 days
(i.e., about 336 hours, about 168 hours, about 84 hours or
about 60 hours or less) before administration of the
platinum-containing chemotherapeutic agent, loop diuretic
agent, etc., or exposure to noise or radiation.  Likewise,
subsequent administration of the effective amount of the
otoprotective agent can be conducted broadly within the
period including as much as 14 days (i.e., including about
60 hours, about 84 hours, about 168 hours or about 336 hours
or more) after administration of the platinum-containing
chemotherapeutic agent, loop diuretic agent, etc., or
exposure to noise or radiation.
Preferably, prior administration of the effective
amount of the methionine protective agent is within about 48
hours before administration of the platinum-containing

chemotherapeutic agent, loop diuretic agent, etc., or
exposure to noise or radiation; with subsequent
administration within about 48 hours after administration of
the platinum-containing chemotherapeutic agent, loop
diuretic agent, etc., or exposure to noise or radiation.
More preferably, prior administration can be within about 36
hours before administration of the platinum-containing
chemotherapeutic agent, loop diuretic agent, etc., or
exposure to noise or radiation; and subsequent
administration can be within about 36 hours after
administration of the platinum-containing chemotherapeutic
agent, loop diuretic agent, etc., or exposure to noise or
radiation.  Still more preferably, prior administration can
be within about 25 hours before, and subsequent
administration can be within about 25 hours after,
administration of the platinum-containing chemotherapeutic
agent, loop diuretic agent, etc., or exposure to harmful
levels of noise or radiation.  Even more preferably, prior
administration can be within about 6 hours before, and
subsequent administration can be within about 1 hour after,
administration of the platinum-containing chemotherapeutic
agent, loop diuretic agent, etc., or exposure to noise or
radiation.  Even more preferably, prior administration of
the effective amount of methionine protective agent can be
within about 1 hour before, and subsequent administration
can be within about 1 hour after, administration of the
platinum-containing chemotherapeutic agent, loop diuretic
agent, etc., or exposure to noise or radiation.  Still more
preferably, prior administration of the effective amount of
methionine protective agent can be within about one-half
hour before, and subsequent administration can be within
about one-half hour after, administration of the platinum-
containing chemotherapeutic agent, loop diuretic agent,
etc., or exposure to noise or radiation.

The platinum-containing chemotherapeutic agent can be administered parenterally, for example by slow intravenous infusion, or by local injection, as discussed above. Loop diuretic agents can be administered orally or parenterally, for example by slow intravenous infusion or by local injection, as is well known in the art. Aminoglycoside antibiotics, iron chelating agents, quinine, quinidine, and radiation can also be administered by routes and in doses conventional in the art. The methionine protective agent can be administered orally, parenterally by intravenous injection or slow infusion, intraperitoneally or topically to the round window membrane.

In a preferred embodiment of the present invention, when treating or preventing otoxicity due to exposure to noise, the effective amount of the otoprotective agent can be administered prior to, simultaneously with, or subsequently to the noise exposure. For example, it has been found that administering the otoprotective agent twice daily for a period ranging from about 14 days (i.e., from about 336, 168, 84, or 60 hours or less) before the noise exposure to about 14 days (i.e., from up to about 60, 84, 168, or 336 hours) after the noise exposure, preferably from about 7 days before the exposure to noise to about 7 days after the exposure to noise, and more preferably from about 48 hours before the exposure to noise to about 48 hours after the exposure to noise (i.e., from about 36, 24, 12, 6, 4, 2, 1 or ½ hour before to about ½, 1, 2, 4, 6, 12, 24 or 36 hours after the exposure to noise), can significantly ameliorate or prevent ototoxicity in a human or animal patient.

When the otoprotective agent is administered to the round window membrane, the otoprotective agent may be administered continuously or periodically during any of the time periods described above by introducing a topical

solution into the ear as described above, i.e., by
administration with a micro-catheter or by injection into
the middle ear, preferably by administration with a micro-
catheter equipped with an automatic, battery-operated pump.

5          Delayed toxic effects due to platinum-containing
chemotherapeutic agents have been observed.  The protective
effects of the present methionine protective agents can be
enhanced by administering them in a supplemental manner
during the course of the patient's chemotherapy and/or

10    afterwards as necessary or as desired.  The same
considerations apply in the case of loop diuretics,
aminoglycoside antibiotics, iron chelating agents, quinine,
quinidine, noise-induced hearing damage, and radiation
exposure.  Thus, the methods described herein can further

15    comprise semi-daily, daily or weekly administration of a
supplemental amount of methionine protective agent.

          Stated another way, it is often beneficial to
administer supplemental doses of the otoprotective agents of
the present invention so as to maintain effective blood

20    serum levels of the otoprotective agents.  Generally, the
administration of supplemental amounts of otoprotective
agents should result in the blood serum level of the human
or animal patient being maintained within at least about
10%, preferably from about 20% to about 70%, and more

25    preferably within about 40%, of the blood serum level of the
patient that results from the administration of the
effective amount of otoprotective agent.  Typically, such
supplemental doses are administered within the time frames
and dosages set forth above for the effective amount of

30    otoprotective agents, for example, semi-daily, daily or
weekly for a period of from about one to fourteen days after
the administration of the effective amount.

          As with the effective amount of methionine protective
agent described above, the supplemental methionine

protective agent can be administered orally; parenterally by
intravenous injection or slow infusion; intraperitoneally or
topically by application to the round window membrane.  When
administered parenterally, the supplemental amount of the
methionine protective agent is preferably in the range of
from about 1.0 mg/kg body weight to about 600 mg/kg body
weight, more preferably from about 5 mg/kg body weight to
about 500 mg/kg body weight, even more preferably from about
10 mg/kg body weight to about 400 mg/kg body weight.

        Alternatively, the supplemental amount of methionine
protective agent parenterally administered daily or weekly
can be expressed on a mole:mole basis in relation to the
anti-cancer effective amount of platinum-containing
chemotherapeutic agent.  This effective amount can be in the
range of from about 4:1 to about 167:1, more preferably from
about 4.25:1 to about 100:1, and most preferably from about
4.68:1 to about 20:1, methionine protective agent:platinum-
containing chemotherapeutic agent, on a molar basis.  A
dosing ratio of about 18.75:1 on a molar basis is preferred.

        Oral or parenteral doses administered daily can be
within the lower ranges listed above.  When administered
orally, daily or weekly doses should be designed to achieve
serum levels equivalent to those achieved by administration
of the various parenteral doses described above.

        When administered topically to the round window
membrane of the ear, the supplemental amount of
otoprotective agent may be administered in the same way as
described above for the effective amount, typically  as a
pharmaceutical formulation such as a topical solution.
Generally, the supplemental topical administration comprises
applying from about 0.001 ml to about 0.010 ml, preferably
from about 0.0025 ml to about 0.0075 ml, and most preferably
about 0.005 ml of a topical solution comprising the
otoprotective agent to the round window membrane.  The

topical solution typically comprises from about 10 mg/ml to
about 50 mg/ml, preferably from about 20 mg/ml to about 30
mg/ml, and most preferably about 25 mg/ml of otoprotective
agent.

## Optimization of Treatment Regimen

In the methods of preventing or reducing ototoxicity of
the present invention, various parameters associated with
the patient's hearing and vestibular systems can be tested
by methods well known in the art to establish pretreatment
baseline values.  After administration of the methionine
protective agent, and over the course of chemotherapy and
afterwards, ototoxic effects can be monitored by
conventional tests, and the results can be compared to those
obtained prior to treatment to determine if any change has
occurred.  If any impairment is observed, the amount and/or
time of administration of the protective agent administered
in conjunction with subsequent doses of the platinum-
containing chemotherapeutic agent, loop diuretic agent,
aminoglycoside antibiotic, iron chelating agent, quinine,
quinidine, or exposure to noise or radiation, can be
adjusted so as to reduce or prevent further ototoxic changes
without substantially diminishing the antineoplastic
effectiveness of the platinum-containing chemotherapeutic
agent or radiation, the diuretic effect of the loop diuretic
agent, etc.  Similar modification of treatment parameters in
the case of weight loss, gastrointestinal toxicity due to
either the platinum-containing chemotherapeutic agent or
radiation, neurotoxicity due to either the platinum-
containing chemotherapeutic agent or radiation, alopecia due
to either the platinum-containing chemotherapeutic agent or
radiation, and overall patient condition/survival due to
either the platinum-containing chemotherapeutic agent or
radiation can be employed to optimize the protective effects

of the protective agent with respect thereto. This can be achieved via appropriate testing and comparison of pre- and post-treatment values, e.g., patient weight and patient physical/medical/physiological condition, etc., with

5    protocol adjustments being made as needed.

The invention being thus described, it will be obvious that the same can be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications

10   and equivalents as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

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